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Altered temporal lobe white matter lipid ion profiles in an experimental model of sporadic Alzheimer's disease☆

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ABSTRACT

Background: White matter is an early and important yet under-evaluated target of Alzheimer's disease (AD). Metabolic impairments due to insulin and insulin-like growth factor resistance contribute to white matter degeneration because corresponding signal transduction pathways maintain oligodendrocyte function and survival.
Methods: This study utilized a model of sporadic AD in which adult Long Evans rats administered intracerebral streptozotocin (i.c. STZ) developed AD-type neurodegeneration. Temporal lobe white matter lipid ion profiles were characterized by matrix-assisted laser desorption/ionization-imaging mass spectrometry (MALDI-IMS).
Results: Although the lipid ion species expressed in the i.c. STZ and control groups were virtually identical, i.c. STZ mainly altered the abundances of various lipid ions. Correspondingly, the i.c. STZ group was distinguished from control by principal component analysis and data bar plots. i.c. STZ mainly reduced expression of lipid ions with low *m/z*'s (less than 810) as well as the upper range *m/z* lipids (*m/z* 964–986), and increased expression of lipid ions with *m/z*'s between 888 and 937. Phospholipids were mainly included among the clusters inhibited by i.c. STZ, while both sulfatides and phospholipids were increased by i.c. STZ. However, Chi-Square analysis demonstrated significant i.c. STZ-induced trend reductions in phospholipids and increases in sulfatides ($P < 0.00001$).
Conclusions: The i.c. STZ model of sporadic AD is associated with broad and sustained abnormalities in temporal lobe white matter lipids. The findings suggest that the i.c. STZ model could be used for pre-clinical studies to assess therapeutic measures for their ability to restore white matter integrity in AD.

1. Introduction

Alzheimer's disease (AD) is manifested by progressive behavioral changes, loss of recent memory, and declines in executive and cognitive functions (McKhann et al., 2011). The characteristic neuropathological changes in AD include brain accumulations of hyper-phosphorylated tau (pTau)-containing cytoskeletal lesion, and increased amyloid-beta (Aβ42) deposits in plaques, vessels, and neurons (Kalaria and Ballard, 1999; Viola and Klein, 2015). However, other more extensive and universal pathologies, including atrophy of white matter (WM), loss of neurons and synaptic terminals, neuro-inflammation, reactive astrocytosis, micro-vascular disease, and increased cellular stress with activation of the unfolded protein response (de la Monte, 2016; Hyman et al., 2012; Montine et al., 2012; Nelson et al., 2012) have received relatively little attention. Failure to attend to the full spectrum of disease could account for the persistent difficulties in rendering accurate diagnoses and repeated failure of clinical trials designed to treat just one aspect of AD (de la Monte, 2016).

WM atrophy and degeneration in AD was first characterized in 1986 by Brun and Englund (Brun and Englund, 1986a, 1986b) and subsequently shown to represent an early abnormality that emerged in pre-clinical stages of disease (de la Monte, 1989). Its main histopathological features include pallor of myelin staining with Luxol fast blue dye due to

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decreased myelin density, attrition or rarefaction of axons, reduced populations of oligodendrocytes, reactive gliosis (scarring), and vascular degeneration (Brickman et al., 2008; Brilliant et al., 1995; Brun and Englund, 1986a; Burns et al., 2005; de la Monte, 1989; Englund, 1998; Sjobeck and Englund, 2003; Sjobeck et al., 2005). In addition, WM atrophy in AD has been linked to increased accumulation of $A\beta_{1-40}$ and $A\beta_{1-42}$ (Roher et al., 2002).

WM is largely composed of lipid-rich myelin sheaths that are synthesized and maintained by oligodendrocytes. The wrapping of myelin sheaths around axons provides insulation, ensuring efficient electrochemical conductivity. Impaired function or death of oligodendrocytes disrupts myelin homeostasis, leading to myelin loss and compromised neurotransmission, plasticity and cognition. Mechanistically, myelin breakdown could be mediated by increased susceptibility of oligodendrocytes to free radical and other types of metabolic injury (Bartzokis, 2004).

Major CNS WM lipids include cholesterol, glycosphingolipids, i.e. cerebrosides (galactosylceramide, galactocerebroside), sulfatides (sulfated galactocerebroside, sulfogalactosylceramide), gangliosides, and phospholipids, consisting of glycerophospholipids (phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS) and plasmalogens) and sphingomyelin (Quarles et al., 2006). Sphingomyelin is composed of ceramide plus a phosphocholine or phosphoethanolamine polar head group (Quarles et al., 2006). Abnormal metabolism and expression of various lipids, including phospholipids and sulfatides occur in a broad range of CNS diseases (Takahashi and Suzuki, 2012). In AD, WM cholesterol, fatty acids, myelin basic protein, and myelin proteolipid protein levels are reduced (Roher et al., 2002; Wang et al., 2004) and sulfatides in both gray and WM are substantially diminished (Han et al., 2002).

Although the mechanisms and consequences of aberrant myelin lipid expression are not well understood, some effects can be predicted based on specific lipid functions. For example, the impairments in intracellular signaling associated with membrane phospholipid deficiencies correlate with their regulatory functions in lipid rafts and membrane receptors. Sulfatides, localized on the extracellular leaflet of myelin plasma membranes and synthesized through sulfonation of galactocerobroside (Vos et al., 1994), regulate neuronal plasticity, memory, myelin maintenance, protein trafficking, adhesion, glial-axonal signaling, insulin secretion, and oligodendrocyte survival (Takahashi and Suzuki, 2012; Vos et al., 1994). Correspondingly, reductions in membrane sulfatide disrupt myelin sheath structure and function, and compromise neuronal conductivity (Kolesnick and Kronke, 1998). Sulfatide degradation via increased galactosylceramidase, sulfatidase or aryl sulfatase activities yields ceramides (Eckhardt, 2008; Sundaram et al., 1995; Vos et al., 1994) that promote neuroinflammation, reactive oxygen species formation, apoptosis, and dysregulated signaling through cell survival and metabolic pathways (Kolesnick and Kronke, 1998).

Despite abundant information about AD's adverse effects on WM, the biochemical nature of its degeneration has not been well characterized due to the lack of suitable tools for efficiently studying pathologic alterations in lipid-rich myelin. Fortunately, over the past several years, major advances in technology and computational science have facilitated extension of Matrix Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI-IMS) to human research. MALDI-IMS is used for in situ imaging of lipids, proteins, and adducts for correlation with histopathology and molecular pathology (in situ hybridization and immunohistochemistry) (Caprioli et al., 1997). Instruments equipped with an Nd:YAG Smartbeam laser enable time of flight (TOF; *m/z*) analysis for specific identification of molecules (Yalcin and de la Monte, 2015).

For this research, we utilized MALDI-IMS to characterize temporal lobe WM myelin lipid profile abnormalities in an intracerebral Streptozotocin (i.c. STZ) model of neurodegeneration. Although STZ is best known for its toxic effects on beta cells in pancreatic islets and production of Type 1 diabetes mellitus, at low doses it causes Type 2 diabetes with peripheral insulin resistance (Bolzan and Bianchi, 2002; Koulmanda et al., 2003; Wang et al., 2011; Yan et al., 2008), and when administered i.c., it causes AD-type neurodegeneration without damaging pancreatic islets or reducing pancreatic production of insulin (Blass et al., 2002; Blum-Degen et al., 1995; de la Monte et al., 2006; de la Monte et al., 2017; Gasparini et al., 2002; Lester-Coll et al., 2006). The rationale for employing the i.c. STZ model is that substantial evidence indicates that the major early abnormalities in AD include reductions in brain glucose metabolism, insulin/IGF trophic factor levels, and insulin/IGF-1 signaling through phosphoinositol-3-kinase (PI3K)-Akt pathways that regulate cell survival, energy metabolism, neuronal plasticity and WM integrity (de la Monte and Tong, 2014; de la Monte and Wands, 2008; Rivera et al., 2005; Steen et al., 2005; Talbot et al., 2012). Regarding WM, oligodendrocyte survival and function are regulated by insulin and insulin-like growth factor type 1 (IGF-1) signaling (Barres et al., 1993; Carson et al., 1993; Chesik et al., 2008) and brain diseases linked to insulin and IGF-1 trophic factor deficiencies or receptor resistances are associated with WM pathology (de la Monte, 2009, 2012, 2016; de la Monte et al., 2009). The i.c. STZ model is widely used because it produces neurobehavioral, histopathological, molecular, and biochemical abnormalities that mimic most aspects of sporadic AD, including amyloid-β deposition, pTau accumulations, cortical-limbic pathway degeneration, deficits in spatial learning and memory, neuroinflammation, increased oxidative stress and WM degeneration (Akinola et al., 2015; de la Monte and Wands, 2008; Paidi et al., 2015; Salkovic-Petrisic et al., 2013; Tong et al., 2016a; Wang et al., 2014).

Table 1
Differential expression of white matter lipids in brains of i.c. STZ-treated rats.

m/z	Lipid identification	Differential expression
702.93	1) PS(30:2); 2) PS(P-31:1)	Control
766.46	PE(38:4)	i.c. STZ
768.48	PE(38:3)	i.c. STZ
796.77	ST(34:0)(OH) Carter	i.c. STZ
832.76	PS(40:7)	i.c. STZ
852.76	PS(42:11)	i.c. STZ
931.76	1) PI(41:2); 2) PI(42:9)	i.c. STZ
988.75	Not identified (unknown)	i.c. STZ

Lipids differentially expressed in only control or only i.c. STZ temporal lobe white matter. Lipid assignments were made using the LIPIDMAPS database or based on previously published MS/MS data.

2. Methods

2.1. Experimental model

A sporadic AD model was generated in 4-week old male Long Evans rats (8/group) by administration of i.c. STZ under ketamine/xylazine anesthesia (de la Monte et al., 2017; Lester-Coll et al., 2006; Tong et al., 2016a; Tong et al., 2016b). Control rats were given i.c. saline. The

Table 2
Temporal lobe white matter lipid composition.

Lipids	Number (%) identified
All sulfatides	39 (24.7%)
Sulfatides	27 (69.2%)
C13-sulfatides	12 (30.8%)
All ceramides	3 (2.0%)
Ceramides	2 (67.0%)
C13-ceramides	1 (33.0%)
All phospholipids	86 (54.4%)
Phospholipids	61 (70.9%)
C13-phospholipids	25 (29.1%)
Not identified	30 (19.0%)

Temporal lobe WM lipids were evaluated by MALDI-IMS and lipid assignments were made using the LIPIDMAPS database or MS/MS results in previous publications.